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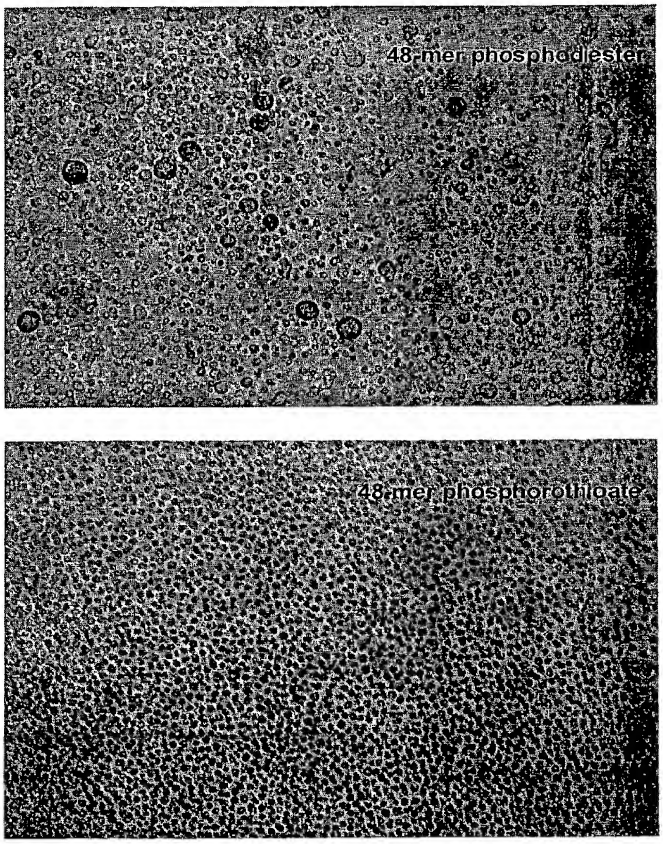

(57) Abstract: The present invention relates to antiviral compositions, agents, and methods of use. In particular, a phosphorothioate oligonucleotide with an optimized chain length is presented. Such phosphorothioate oligonucleotides have a chain length of at least 32 nucleotides and may be further optimized by having about 37 oligonucleotides. The phosphorothioate oligonucleotides of the present invention may be used in methods of inhibiting HIV activity, including the activity of HIV-1 and HIV-2.

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ANTIVIRAL PHOSPHOROTHIOATE OLIGONUCLEOTIDES

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to antiviral agents and method of use. More specifically, the present invention relates to oligonucleotides that are potent inhibitors of retroviral replication.

2. Technical Background

Acquired immune deficiency syndrome (AIDS) was first reported in the United States in 1981 and has since become a major worldwide epidemic. AIDS is caused by the human immunodeficiency virus (HIV). HIV is a retrovirus which infects humans and other primates. HIV has a single stranded RNA genome which requires for its replication the use of an enzyme known as a reverse transcriptase. By killing or impairing cells of the immune system, HIV progressively destroys the body's ability to fight infections and certain cancers. Individuals diagnosed with AIDS are subject to opportunistic infections caused by microbes that usually do not cause illness in healthy people.

More than 600,000 cases of AIDS have been reported in the United States since 1981, and as many as 900,000 Americans may be infected with HIV. HIV is spreading most rapidly among minority populations and is a leading killer of African-American males. According to the U.S. Centers for Disease Control and Prevention (CDC), the prevalence of AIDS is six times higher in African-Americans and three times higher among Hispanics than among whites.

HIV may be spread by a number of different means generally including the transmission of a bodily fluid such as blood or semen. HIV is spread most commonly by sexual contact with an infected partner. HIV is also spread through contact with infected blood, such as through blood transfusion, needle sharing among intravenous drug users, and needle sticks to a health care worker. HIV can also be transmitted from mother to child during pregnancy or birth. Approximately one-quarter to one-third of all untreated pregnant women infected with HIV will pass the infection to their babies. HIV also can be spread to babies through the breast milk of their infected mothers.

Education and treatment methods have shown some potential for the slowing of HIV infections. For example, the practice of "safe sex" such as using condoms or having only one sexual partner has been shown to significantly reduce the risk of obtaining HIV through sexual transmission. Additionally, blood screening and treatment methods have essentially eliminated the risk of contracting HIV through a blood transfusion. Drugs called reverse transcriptase inhibitors such as AZT also reduce the chance of a mother transmitting HIV to her baby.

The initial HIV infection may go unnoticed due to lack of early symptoms. For example, frequently no symptoms are seen when a person first becomes infected with HIV. However, some people have a flu-like illness within a month or two after exposure to the virus. They may have fever, headache, malaise, and enlarged lymph nodes. These symptoms usually disappear within a week to a month and are often mistaken for those of another viral infection. People are very infectious during this initial period, and HIV is present in large quantities in genital secretions increasing the chances of HIV transmission.

A long period of asymptomatic HIV infection generally follows any initial symptoms. This period may be as short as a few months, but is generally a period of years up to ten years. During the asymptomatic period, HIV is actively multiplying and infecting and killing immune cells. This may be seen in a decline in the blood levels of CD4+T cells.

As the immune system is progressively destroyed by HIV, a number of complications and symptoms begin to develop. Such complications may include swollen glands, lack of energy, weight loss, fever, yeast infections, and the like.

The term AIDS refers to the most advanced stage of HIV infection. AIDS refers to a person infected with HIV and who shows one or more serious, life threatening conditions. Generally AIDS applies to a person who has a CD4+T cell count of less than 200. People in the advanced stages of AIDS may have infections from opportunistic organisms such as bacteria, viruses, and fungi which do not usually cause disease in healthy persons. Additionally, people with AIDS are particularly prone to developing various cancers, especially those caused by viruses such as Kaposi's sarcoma and cervical cancer, or cancers of the immune system known as lymphomas.

Currently, a number of drugs are available for the treatment of HIV. These drugs are targeted toward specific enzymes and nucleic acids which are required for viral replication, assembly, or infection. A first group of drugs used to treat HIV infection, called nucleoside analog reverse transcriptase inhibitors (NRTIs), interrupt an early stage of virus replication. Included in this class of drugs are zidovudine (also known as AZT), zalcitabine (ddC), didanosine (ddI), stavudine (D4T), lamivudine (3TC) and abacavir succinate. These drugs may slow the spread of HIV in the body and delay the onset of opportunistic infections. However, they do not prevent

transmission of HIV to other individuals. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) such as delavirdine, nevirapine and efavirenz are also available for use in combination with other antiretroviral drugs. Another class of anti-HIV drugs, called protease inhibitors, interrupts virus replication at a later step in its life cycle. They include ritonavir, saquinivir, indinavir and nelfinavir. Because HIV can become resistant to each class of drugs, cocktail treatments combining drugs from each class are necessary to effectively suppress the virus. Moreover, antiviral drug resistance has emerged to all approved anti-HIV drugs. Pillay et al., *Rev. Med. Virol.* 10:231-53 (2000). Recently, one group of researchers determined by simulation that most of available anti-HIV drugs will become ineffective or less effective in 5 to 10 years due to the emergence of drug resistant HIV strains. Blower et al., *Nat. Med.* 7:1016-20 (2001). Therefore, there is an urgent need to develop new anti-HIV drugs to combat this worldwide pandemic.

Currently available antiretroviral drugs do not cure people of HIV infection or AIDS. In addition al such drugs have side effects that can be severe. AZT may cause a depletion of red or white blood cells, especially when taken in the later stages of the disease. If the loss of blood cells is severe, treatment with AZT must be stopped. DdI can cause an inflammation of the pancreas and painful nerve damage. Other common side effects associated with protease inhibitors include nausea, diarrhea and other gastrointestinal symptoms. In addition, protease inhibitors can interact with other drugs resulting in serious side effects. Moreover, cases of abnormal redistribution of body fat have been reported among some individuals receiving protease inhibitors. Additionally, strains of HIV resistant to one or more of the available drugs are beginning to be isolated from patients with HIV.

Recently, other compounds for treating HIV are being investigated. Many of these compounds show significant anti-HIV activity in the laboratory, but have not proven to be useful when administered to a patient. Many compounds require a high concentration of the compound to provide substantial anti-HIV activity. These high concentrations are difficult if not impossible to achieve and maintain within the serum of a person because the body either metabolizes them or they are cleared from the blood.

For example, oligonucleotides have been proposed as anti-HIV agents. These anit-HIV oligonucleotides are mainly used as antisense oligonucleotides which can hybridize to HIV RNA and inhibit viral protein production. Phosphorothioate oligonucleotides may have a variety of possible non-antisense mechanisms by which they inhibit HIV infection. For example, it has been reported that phosphorothioate oligonucleotides inhibit the adsorption or penetration of HIV into cells. Goa et al., Molecular Pharmacology 41:223-229 (1992). It has also been postulated that the specific interaction of phosphorathioates with the HIV-1 V3 loop may inhibit HIV-1 entry into cells. Stein et al., U.S. Pat. No. 5,756,710. A guanosine-rich oligonucleotide having a tetrad structure which inhibits HIV integrase has also been proposed. Rando et al., U.S. Pat. No. 6,323,185. The potential mechanism by which phosphorothioate oligodeoxycytidine directly inhibits viral reverse transcriptase has also been proposed. Marshall et al., Proc. Natl. Acad. Sci. USA 89:6265-6269 (1992). It has been reported that the maximal anti-HIV activity of phosphorothioate oligonucleotides is at 21 to 28 nucleotides in length and the maximal anti-HIV activity is achieved at 1-2 micromolar concentrations. Stein, et. al., *AIDS Res. Hum. Retroviruses* 5:639-646 (1989) Moreover, it has also been reported that a discrete increase in binding constant of phosphorothioate oligonucleotide to HIV surface

glycoprotein gp120 is observed as the chain length of the oligonucleotides increase. However, the binding constant of phosphorothioate oligonucleotides was not increased as the chain length for a chain length longer than 18 nucleotides. Stein, et. al., *Antisense Res. Dev.* 3:19-31 (1993). It was also reported that the addition of SdG_4 and other phosphorothioate motifs (sequences) did not significantly augment the anti-HIV potency of 28-mer phosphorothioate oligonucleotides. Stein, et al., *Antisense Res. Dev.* 6:281-289 (1996). However, due to the poor efficacy of anti-HIV phosphorothioate oligonucleotides tested to date, it has not been possible to develop these compounds as viable anti-HIV therapeutics.

Accordingly, it would be an advancement in the art to provide an antiviral compound that inhibits the replication of a retrovirus. It would be an additional advancement to provide such a compound which could be used to inhibit the replication of HIV. It would be a further advancement if the compound were active at low concentrations. It would be a further advancement if the compound could treat a person infected with HIV. Such compounds are disclosed and claimed herein.

BRIEF SUMMARY OF THE INVENTION

The present invention relates to an antiviral composition for inhibiting the replication of retroviruses. The composition can be used to treat infections of such retroviruses as human immunodeficiency virus. The composition contains an effective amount of a phosphorothioate oligonucleotide which has been selected for optimal chain length for inhibiting the replication the virus. In certain embodiments, chain length of at least 32 nucleotides is sufficient for inhibition of viral replication. An effective amount of a phosphorothioate oligonucleotide of at least 32 nucleotides has been found to effectively inhibit the replication of HIV-1 and HIV-2. More

specifically, a chain length of more than about 37 nucleotides may more effectively inhibit the replication of the retrovirus. A phosphorothioate oligonucleotide having a chain length of at least about 40 nucleotides may also be effective to inhibit replication of a retrovirus. Phosphorothioate oligonucleotides, having a chain length of from about 34 to about 50 nucleotides may also be used for retrovirus inhibition.

The sequence of such antiviral oligonucleotides may vary so long as the optimal chain length of the phosphorothioate oligonucleotide in maintained. For example, phosphorothioate oligonucleotides having an optimal chain length, but with the sequence of SEQ. ID. NO.: 4, SEQ. ID. NO.: 5, SEQ. ID. NO.: 6, SEQ. ID. NO.: 7, SEQ. ID. NO.: 8, SEQ. ID. NO.: 9, SEQ. ID. NO.: 10, SEQ. ID. NO.: 11, SEQ. ID. NO.: 12, SEQ. ID. NO.: 13, SEQ. ID. NO.: 14, SEQ. ID. NO.: 15, SEQ. ID. NO.: 16, SEQ. ID. NO.: 17, SEQ. ID. NO.: 18, SEQ. ID. NO.: 19, or SEQ. ID. NO.: 20 have been shown to effectively inhibit the replication of a retrovirus.

The antiviral phosphorothioate oligonucleotides of the present invention may have a variety of features and characteristics and yet maintain their antiviral characteristics. For example, the oligonucleotide may have a sequence which creates a 3' hairpin structure. Such antiviral phosphorothioate oligonucleotides having a 3' hairpin structure may have the sequence of SEQ. ID. NO.: 10, SEQ. ID. NO.: 11, SEQ. ID. NO.: 12, SEQ. ID. NO.: 13, SEQ. ID. NO.: 14, SEQ. ID. NO.: 15, SEQ. ID. NO.: 16, SEQ. ID. NO.: 17, or SEQ. ID. NO.: 18.

Other structures may contribute to the antiviral qualities of the phosphorothioate oligonucleotide such as a 3' inverted thymine base. Phosphorothioate oligonucleotides with a 3' inverted thymine base such as SEQ. ID. NO.: 10, SEQ. ID. NO.: 11, SEQ. ID. NO.: 12, SEQ. ID. NO.: 13, SEQ. ID. NO.: 14, SEQ. ID. NO.: 15, SEQ. ID. NO.: 16, SEQ. ID. NO.: 17, SEQ. ID. NO.: 18, SEQ. ID.

NO.: 19, and SEQ. ID. NO.: 20 have been shown to inhibit the replication of a retrovirus.

The antiviral composition of the present invention may be used to inhibit syncytia formation caused by a human immunodeficiency virus infection. The human immunodeficiency virus may be contacted with an effective amount of an antiviral composition as described herein. Immunodeficiency viruses that cause formation of syncytia, that may be inhibited by the antiviral composition, include HIV-1 and HIV-2. The antiviral compositions of the present invention are potent inhibitors of human immunodeficiency viruses. Thus, the antiviral compositions may be administered in doses such that a final concentration of the phosphorothioate oligonucleotide in the blood, culture medium, serum, or other fluid containing cells infected by the virus, is in the range from about 5 nM to about 100 nM. A final fluidic concentration of about 10 nM to about 50 nM may be effective at inhibiting syncytia formation. A final fluidic concentration greater than about 10 nM is generally effective in inhibiting syncytia formation. Alternatively, a final fluidic of greater than about 40 nM may be used.

The phosphorothioate oligonucleotides of the present invention may also be used to inhibit the replication of a human immunodeficiency virus. The replication of a human immunodeficiency virus may be inhibited by contacting the human immunodeficiency virus with a phosphorothioate oligonucleotide with a chain length of at least about 32 bases. A phosphorothioate oligonucleotide with a chain length of at least about 37 may also be effective at inhibiting replication of a human immunodeficiency virus. Generally, phosphorothioate oligonucleotides with a chain length in the range from about 34 to about 50 are effective at inhibiting the replication of human immunodeficiency viruses such as HIV-1 and HIV-2.

Phosphorothioate oligonucleotides that inhibit the replication of a human immunodeficiency virus may have the sequence of SEQ. ID. NO.: 5, SEQ. ID. NO.: 6, SEQ. ID. NO.: 7, SEQ. ID. NO.: 8, SEQ. ID. NO.: 9, SEQ. ID. NO.: 10, SEQ. ID. NO.: 11, SEQ. ID. NO.: 12, SEQ. ID. NO.: 13, SEQ. ID. NO.: 14, SEQ. ID. NO.: 15, SEQ. ID. NO.: 16, SEQ. ID. NO.: 17, SEQ. ID. NO.: 18, SEQ. ID. NO.: 19, and SEQ. ID. NO.: 20.

Such anti-human immunodeficiency virus oligonucleotides may have a 3' hair pin loop structure such as the oligonucleotides of SEQ. ID. NO.: 10, SEQ. ID. NO.: 11, SEQ. ID. NO.: 12, SEQ. ID. NO.: 13, SEQ. ID. NO.: 14, SEQ. ID. NO.: 15, SEQ. ID. NO.: 16, SEQ. ID. NO.: 17, and SEQ. ID. NO.: 18.

Additionally, the anti-human immunodeficiency oligonucleotides may have a 3' inverted thymine base such as the oligonucleotides of SEQ. ID. NO.: 10, SEQ. ID. NO.: 11, SEQ. ID. NO.: 12, SEQ. ID. NO.: 13, SEQ. ID. NO.: 14, SEQ. ID. NO.: 15, SEQ. ID. NO.: 16, SEQ. ID. NO.: 17, SEQ. ID. NO.: 18, SEQ. ID. NO.: 19, and SEQ. ID. NO.: 20.

The oligonucleotides of the present invention are potent inhibitors of human immunodeficiency viruses such that the compound may be effective at low final concentration in blood, culture medium, serum, or other fluid in which the inhibition of a human immunodeficiency virus may be desired. Thus, the oligonucleotide may be effective at a final concentration in the desired fluid in the range from about 5 nM to about 100 nM. It has been found that a final concentration of the oligonucleotide in the range from about 10 nM to about 50 nM can be effective at inhibiting the replication of a human immunodeficiency virus. Alternatively a final fluidic concentration of at least about 10 nM may be used. A final fluidic concentration of

the phosphorothioate oligonucleotide of at least about 40 nM may be effective to inhibit viral replication.

BRIEF DESCRIPTION OF THE DRAWINGS

A more particular description of the invention briefly described above will be rendered by reference to specific embodiments thereof which can be understood by reference to the following figures. These figures depict only typical embodiments of the invention and are not therefore to be considered to be limiting of its scope. The invention will be described and explained with additional specificity and detail through the use of the accompanying drawings in which:

Figure 1 is a bar graph showing the length dependent anti-HIV activity of phosphorothioate oligonucleotides.

Figure 2 is a bar graph showing the dose dependent anti-HIV activity for a 39-mer and a 48-mer phosphorothioate oligonucleotide.

Figure 3A is a photograph showing syncytial formation in CEMtart cells infected with HIV1MC99d(tat/rev) and treated with a 48-mer phosphodiester oligonucleotide.

Figure 3B is a photograph showing the absences of syncytial formation in CEMtart cells infected with HIV1MC99d(tat/rev) and treated with a 48-mer phosphorothioate oligonucleotide.

DETAILED DESCRIPTION

It will be readily understood that the components of the present invention, as generally described and illustrated in the figures herein and specification, could be designed in a wide variety of different configurations. Thus, the following more detailed description of the embodiments of the composition and method of the present invention, as represented in Figures 1 through 4, is not intended to limit the scope of

the invention, as claimed, but is merely representative of presently preferred embodiments of the invention.

The present invention relates to a antiviral compositions for inhibiting the activity of a retrovirus, particularly a human immunodeficiency virus (HIV). The term HIV includes, but is not limited to HIV-1 and HIV-2. The HIV activity that may be inhibited by the antiviral composition of the present invention includes replication, infectivity, cell-cell fusion, the formation of multinucleated giant cells, and the formation of heterokaryons. Such HIV activity may be measured by, for example, measuring p24 production, measuring reverse transcriptase activity, or measuring viral load.

The antiviral composition of the present invention contains oligonucleotides which exhibit antiretroviral activity. An oligonucleotide is a molecule comprised of two or more deoxyribonucleotides or ribonucleotides. A nucleotide includes both the deoxyribonucleic acids and ribonucleic acids. Unless otherwise noted standard abbreviations are used to refer to nucleic acid molecules and other chemicals. "A" refers to adenosine as well as to its deoxyribose derivative. "T" refers to thymidine as well as to its deoxyribose derivative. "G" refers to guanosine as well as its deoxyribose derivative. "C" refers to cytidine as well as its deoxyribose derivative. The nucleotides disclosed herein may be modified or derivatized by methods known in the art. Additionally, nucleotides can refer to other natural and synthetic nucleotides such as inosine, deoxyinosine, hypoxanthine, isosteric purine 2'deoxy-furanoside analogues, 2'-deoxynebularine or 2'deoxyxanthosine, or other purine and pyrimidine analogues.

The composition of the present invention contains an effective amount of a phosphorothioate oligonucleotide which has been selected for an optimal chain length

for inhibiting the replication of the virus. The term chain length refers to the number of joined nucleotide units that form the oligonucleotide. Optimal chain length means a nucleotide chain length of an oligonucleotide that produces the greatest antiviral activity. A significant anti-HIV activity has been observed with a phosphorothioate oligonucleotide having a chain length of at least 32. However, the anti-HIV activity of the phosphorothioate oligonucleotides of the present invention increased dramatically when the chain length was increased to greater than 34 nucleotides. Complete inhibition of HIV activity was observed when the chain length was extended to greater than 36 nucleotides. Thus, a minimum chain length of about 37 can essentially completely inhibit HIV activity.

A phosphorothioate oligonucleotide refers to an oligonucleotide having one or more phosphorothioate inter-sugar linkages. A phosphorothioate oligonucleotide refers to a phosphorothioate oligodeoxynucleotide, a phosphorodithioate, a chimeric oligonucleotide, or a phosphorothioate oligonucleotide which is further linked to another chemical moiety. Phosphorothioate oligonucleotide may also include an oligonucleotide or oligodeoxynucleotide in which sulfur replaces one or more of the non-bridging oxygen atoms in one or more phosphodiester linkages. Each phosphorothiodiester linkage can occur as either an Rp or Sp diastereomer. A bridging oxygen atom is an oxygen atom in a phosphodiester linkage of a nucleic acid which joins phosphorous to a sugar.

One or more of the phosphorothiodiester linkages of the phosphorothioate oligonucleotide moiety may be modified by replacing one or both of the two bridging oxygen atoms of the linkage with analogues such as --NH, --CH2, or --S. Other oxygen analogues known in the art may also be used.

A phosphorothioate oligonucleotide may be stereo regular, stereo non-regular or stereo random. A stereo regular phosphorothioate oligonucleotide is a phosphorothioate oligonucleotide in which all the phosphodiester linkages or phosphorothiodiester linkages polarize light in the same direction. Each phosphorous in each linkage may be either a Sp or Rp diastereomer. Generally, synthetic phosphorothioate oligonucleotides which synthesizer are stereo random which means that each phosphorous atom in the phosphorothioate oligonucleotide has a 50% chance of being either a Sp or Rp diastereomer.

The antiviral composition can also include a pharmaceutically acceptable carrier or other molecule that enhances the antiviral characteristics of the phosphorothioate oligonucleotide. Thus, the phosphorothioate oligonucleotide may be administered to cell culture, a patient, or an animal model by itself or in combination a carrier molecule. Suitable carrier molecules can be any of the standard pharmaceutically accepted carriers known to those of ordinary skill in the art such as phosphate buffered saline solution, water, emulsions such as oil/water emulsions or a triglyceride emulsion, various types of wetting agents, tablets, coated tablets and capsules. A suitable pharmaceutically acceptable carrier may be selected taking into account the chosen mode of administration and the characteristics of the infected subject. The phosphorothioate oligonucleotides of the present invention may be combined with other drugs and compounds that are used to treat HIV and HIV related disorders. These drugs and compound may include antibodies, peptides, protease inhibitors, reverse transcriptase inhibitors, vaccines, and a combination thereof.

The sequence of such antiviral oligonucleotides may vary so long as the optimal chain length of the phosphorothioate oligonucleotide is maintained. For example, phosphorothioate oligonucleotides having an optimal chain length, but with

the sequence of SEQ. ID. NO.: 4, SEQ. ID. NO.: 5, SEQ. ID. NO.: 6, SEQ. ID. NO.: 7, SEQ. ID. NO.: 8, SEQ. ID. NO.: 9, SEQ. ID. NO.: 10, SEQ. ID. NO.: 11, SEQ. ID. NO.: 12, SEQ. ID. NO.: 13, SEQ. ID. NO.: 14, SEQ. ID. NO.: 15, SEQ. ID. NO.: 16, SEQ. ID. NO.: 17, SEQ. ID. NO.: 18, SEQ. ID. NO.: 19, or SEQ. ID. NO.: 20 have been shown to effectively inhibit the replication of a retrovirus. These phosphorothioate oligonucleotides can include other features and characteristics that enhance or contribute to their antiviral properties. For example, the oligonucleotide may have a 3' inverted thymine base. Additionally, antisense phosphorothioate oligonucleotides directed toward a viral gene may be used. Phosphorothioate oligonucleotides with a sequence which creates a 3' hairpin structure have also been shown to be potent antiviral agents.

The antiviral composition of the present invention may be used in methods of inhibiting the activity of HIV. Such HIV activity may include the HIV replication, production of viral peptides, formation of syncytia, and other HIV activities. Syncytia are giant cells containing many nuclei not separated by cell membranes. Syncytia are well known to be associated with a HIV infection in a human subject or cell culture.

Such methods of inhibiting HIV activity include contacting HIV with an effective amount of a phosphorothioate oligonucleotide with an optimal chain length. The phosphorothioate oligonucleotide with an optimal chain length may be a component of a pharmaceutical composition containing a suitable carrier, other anti-HIV drugs or compounds which otherwise enhance or facilitate the anti-HIV effect of the optimized phosphorothioate oligonucleotide. Anti-HIV refers a compound, composition, or method that either partly or completely inhibits an HIV infection. Such anti-HIV activity includes, but is not limited to, inhibiting virus entry into cells, inhibiting viral replication, inhibiting production of viral nucleic acids and/or proteins,

inhibiting viral maturation, inhibiting viral budding and release from cells, inhibiting virus induced host physiological and pathological responses.

The phosphorothioate oligonucleotides of the present invention may be used for treating an HIV infection in a human subject. Additionally, the phosphorothioate oligonucleotides may be used to treat an HIV related disorder. Examples of HIV related disorders include, but are not limited to, AIDS, immunodeficiency, central nervous system disease, HIV encephalopathy, neuropathy, pneumocystis, Kaposi's sarcoma, carinii pneumonia, non-Hodgkin's lymphoma, and Hodgkin's lymphoma. Other HIV related disorders are known in the art, and any of these disorders may be treated according to the invented method.

The phosphorothioate oligonucleotides of the present invention may be used to treat an HIV infection no matter how the HIV virus was contracted. Moreover, the phosphorothioate oligonucleotides with optimized chain lengths may be used prophylactically to prevent the infection in a patient who has a high risk for an HIV infection. Such persons who may be at risk for an HIV infection include persons with infected sexual partners, intravenous drug users, healthcare workers, persons receiving blood transfusions, organ recipients, unborn children of infected mothers, etc. Thus, inhibition of HIV includes both prevention and treatment of HIV infection.

An effective amount or effective dose of the phosphorothioate oligonucleotide or composition may vary depending on the desired effect, the size of the subject, the strain of HIV, and the like. The effective amount or does refers to an amount of a compound that achieves the desired result in a cell culture, animal model, or a human patient. It will be appreciated that the amount of the effective dose will vary depending on a number of factors. These factors include, when applicable, the age, sex, height, weight, and diet of the human or animal to be treated, as well as the viral

strain being tested. Generally, a dose of the antiviral composition or phosphorothioate oligonucleotide will be administered to achieve a desired final concentration. The final concentration refers to molar concentration of the phosphorothioate oligonucleotide in a fluid containing viral infected cells. Such fluids may include blood, culture medium, serum, or other fluid which may support the growth of cells by the virus. A final concentration of a phosphorothioate oligonucleotide of the present invention having a chain length of about 39 or 40 nucleotides was shown to dramatically reduce syncytia formation in an HIV infected cell culture at a dose as low as 20 nM. The anti-HIV effect increased with no syncytia formation occurring at a dose between about 40 nM and about 50 nM. A concentration of about 41 nM should be effective to completely inhibit HIV activity in most circumstances.

The antiviral composition containing a phosphorothioate oligonucleotide may be administered to a subject through any known mode of administration. Such modes of administration include, but are not limited to, topical administration, parenteral administration, oral administration, or intraperitoneal, intravenous, intrathecal, intratracheal, intramuscular, or subcutaneous injection. Administration of the phosphorothioate oligonucleotide may be effected continuously or intermittently.

EXAMPLES

The following examples are given to illustrate various embodiments which have been made within the scope of the present invention. It is to be understood that the following examples are neither comprehensive nor exhaustive of the many types of embodiments which can be prepared in accordance with the present invention.

Example 1: Optimization of Nucleotide Chain Length

To determine the effect of the length of a phosphorothioate oligonucleotide on viral replication a set of culture wells were prepared. CEMtart cells (104 cells) were

seeded in 1 ml of RPMI-1640 medium in a culture well. The cells were infected with HIV1MC99d(tat/rev).

The HIV infected CEM tart cells were then treated with 50 nM of phosphorothioate oligonucleotides of various chain lengths. Each of the phosphorothioate oligonucleotides were homopolydeoxycytidine with a chain length of either 24 nucleotides (SEQ. ID. NO.: 1), 26 nucleotides (SEQ. ID. NO.: 2), 28 nucleotides (SEQ. ID. NO.: 3), 30 nucleotides (SEQ. ID. NO.:4), 32 nucleotides (SEQ. ID. NO.: 5), 34 nucleotides (SEQ. ID. NO.: 6), 36 nucleotides (SEQ. ID. NO.: 7), 38 nucleotides (SEQ. ID. NO.: 8), or 40 nucleotides (SEQ. ID. NO.: 9).

On the fifth day post infection, syncytial formation was counted. The cell culture was centrifuged to pellet the cells. The cell pellet was suspended in 50 μl of Trypan Blue. The number of syncytia per culture well was counted under a microscope using a hemocytometer.

As shown in Figure 1, the anti-HIV activity of the phosphorothioate oligonucleotides varied depending on length. The data indicates that a phosporothioate oligonucleotide with a length of 24 nucleotides has minimal anti-HIV effect. The anti-HIV effect increases in a length dependent manner with maximal or total inhibition achieve between about 36 and about 40 nucleotides in length. Fitting of this length-response data indicates that with a minimum chain length of 37 nucleotides a phosphorothioate oligonucleotide, a total inhibition of HIV is achieved. (IC100 in Table 1).

Table 1. Length Response Parameters of Phosphorothioate Oligonucleotides. IC50, IC90, and IC100 denote the chain lengths of phosphorothioate oligonucleotides at which 50%, 90%, and 100% of HIV induced syncytial formation is inhibited respectively. The data indicates that the minimal oligonucleotide chain length for complete inhibition of HIV infection is about 37 nucleotides.

(n=5)	IC50	IC90	IC100
Mean (nucleotides)	17.97	32.78	36.48
Std. Error	0.66	0.56	0.54

Example 2: Effect of Dose of Optimized Phosphorothioate Oligonucleotides

The minimal dose of a phosphorothioate oligonucleotide to produce the highest inhibition of HIV was investigated. CEMtart cells (104 cells) were seeded in 1 ml of RPMI-1640 medium in a culture well. The cells were infected with HIV1MC99d(tat/rev). The cells HIV infected cells were treated with a dose of either a 39-mer (SEQ. ID. No. 10) or a 48-mer (SEQ. ID. NO. 12). The dose of the phosophorothioate oligonucleotide was 10, 20, 30, 40, or 50 nM.

On the fifth day post infection, syncytial formation was counted. The cell culture was centrifuged to pellet the cells. The cell pellet was suspended in 50 μl of Trypan Blue. The number of syncytia per culture well was counted under a microscope using a hemocytometer.

As shown in Figure 2, the anti-HIV activity of phosphorothioate oligonucleotides is dose dependent. There is no significant difference ($P>0.05$) in anti-HIV activity between the 39-mer and 48-mer oligonucleotides at all the concentrations tested. Data fitting (Table 2) indicates that the minimal oligonucleotide concentration for complete inhibition of HIV infection by phosphorothioate oligonucleotides with optimal chain lengths (equal to or greater than 37-mer) is about

41 to 42 nM. Further increasing the optimal chain length does not significantly increase the anti-HIV activity of the phosphorothioate oligonucleotides.

Table 2. Dose Response Parameters of a 39-mer and a 48-mer Phosphorothioate oligonucleotide. IC50, IC90, and IC100 denote the concentrations of the phosphorothioate oligonucleotide at which 50%, 90%, and 100% of HIV induced syncytial formation is inhibited respectively. There is no significant difference between each of the IC50, IC90, and IC100 of the 39-mer and 48-mer oligonucleotide treatment.

		IC50	IC90	IC100
39-mer	Mean (nM)	18.14	37.24	42.02
	Std. Error	1.19	0.68	0.57
48-mer	Mean (nM)	16.69	36.25	41.14
	Std. Error	0.82	0.48	0.61
(n=5)	P	>0.05	>0.05	>0.05

Example 3: Effect of Phosphodiester Oligonucleotide

The anti-HIV effect of an oligonucleotide with a naturally occurring phosphodiester inter-sugar linkage having a chain length similar to its phosphorothioate counterpart was investigated. CEMtart cells (104 cells) were seeded in 1 ml of RPMI-1640 medium in a culture well. The cells were infected with HIV1MC99d(tat/rev) and concurrently treated with 50 nM of a 48-mer phosphodiester and a phosphorothioate oligonucleotides of the same sequence (SEQ. ID. NO. 11 and 12 respectively).

On the fifth day post infection, syncytial formation was photographed and counted. The cell culture was centrifuged to pellet the cells. The cell pellet was suspended in 50 μl of Trypan Blue. The number of syncytia per culture well was counted under a microscope using a hemocytometer. Both untreated and

phosphodiester oligonucleotide treated cultures produced about 7600 syncytia per well, while no syncytia was detected in phosphorothioate oligonucleotide treated culture (Figure 3). Syncytia-free subcultures lasted as long as the phosphorothioate oligonucleotide treatment continued. The results indicate that phosphorothioate linkage is required for the anti-HIV activity of oligonucleotide.

Example 4: Effect of Nucleotide Sequence

The nucleotide sequence dependence on anti-HIV activity of phosphorothioate oligonucleotides was investigated. CEMtart cells (104 cells) were seeded in 1 ml of RPMI-1640 medium in a culture well. The cells were infected with HIV1MC99d(tat/rev) and concurrently treated with 50 nM of phosphorothioate oligonucleotides of various sequences (SEQ. ID. NO. 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, and 20).

On the fifth day post infection, syncytial formation was scored as positive (+) or negative (-). As shown in Table 3, all the phosphorothioate oligonucleotides (37-mer to 48-mer long), regardless of their nucleotide sequences, completely inhibited syncytial formation at 50 nM. Oligonucleotides (SEQ. ID. NO. 8 and 9) are homopolydeoxycytidine (SdC). Oligonucleotides (SEQ. ID. NO. 13, 14, 15, and 16) are antisense oligonucleotides targeted to the mRNA encoding green fluorescence protein (GFP). Oligonucleotides (SEQ. ID. NO. 10 and 18) are sense and antisense oligonucleotides to the HIV tat mRNA. Oligonucleotides (SEQ. ID. NO. 18 and 19) both target to the same HIV tat mRNA, but one with a 3' hairpin (SEQ. ID. NO. 18) and one without the 3' hairpin (SEQ. ID. NO. 19). These results indicate that the anti-HIV activity of phosphorothioate oligonucleotides of 37-mer or longer is sequence and structure independent.

TABLE 3. Phosphorothioate Oligonucleotide Sequence Is Independent of Anti-HIV Activity. The difference in nucleotide sequences as compared to SEQ. ID. NO. 13 is underlined. The nucleotide t_i denotes an inverted t. The data indicates that anti-HIV activity of phosphorothioate oligonucleotides is independent of their nucleotide sequences.

SEQ. ID.NO.	Length (nucleo-tides)	Sequence	Syncytia
13	37	ctcctcgcccttgctcactccagtcgacgcaagtcgt$_i$	-
14	37	ctcctcgcccttgctcactccagtcgacgaaagtcgt$_i$	-
15	37	ctcctcgcccttgctcactccagtcgacatgcgtcgt$_i$	-
16	37	ctcctcgcccttgctcactccagtgccagcaatggct$_i$	-
17	37	ccccccccccccccccccccccccgacgaaagtcgt$_i$	-
8	38	cccccccccccccccccccccccccccccccccccc	-
10	39	cctagactagagccctggaagcatcccgacgcaagtcgt$_i$	-
18	39	ggatgcttccagggctctagtctaggcgacgcaagtcgt$_i$	-
19	39	ggatgcttccagggctctagtctaggcccccccccccct$_i$	-
9	40	cccccccccccccccccccccccccccccccccccccccc	-
20	41	ccccccccccccccccccccccccccccccccccccccct$_i$	-
12	48	aagcgaaagcttggtgatccatttcttgctccagtcgacgaaagtcgt$_i$	-

The present invention may be embodied in other specific forms without departing from its structures, methods, or other essential characteristics as broadly described herein and claimed hereinafter. The described embodiments are to be considered in all respects only as illustrative, and not restrictive. The scope of the invention is, therefore, indicated by the appended claims, rather than by the foregoing description. All changes that come within the meaning and range of equivalency of the claims are to be embraced within their scope.

CLAIMS:

1. A pharmaceutical composition for inhibiting the replication of a retrovirus comprising:

an effective amount of an oligonucleotide comprising at least one phosphorothioate inter-sugar linkage, the oligonucleotide having a chain length of in the range from about 32 nucleotides to about 50 nucleotides.

2. The pharmaceutical composition of claim 1, wherein the retrovirus is a human immunodeficiency virus.

3. The pharmaceutical composition of claim 2, wherein the human immunodeficiency virus is selected from the group consisting of HIV-1 and HIV-2.

4. The pharmaceutical composition of claim 1, wherein the oligonucleotide is selected from the group consisting of SEQ. ID. NO.: 4, SEQ. ID. NO.: 5, SEQ. ID. NO.: 6, SEQ. ID. NO.: 7, SEQ. ID. NO.: 8, SEQ. ID. NO.: 9, SEQ. ID. NO.: 10, SEQ. ID. NO.: 11, SEQ. ID. NO.: 12, SEQ. ID. NO.: 13, SEQ. ID. NO.: 14, SEQ. ID. NO.: 15, SEQ. ID. NO.: 16, SEQ. ID. NO.: 17, SEQ. ID. NO.: 18, SEQ. ID. NO.: 19, and SEQ. ID. NO.: 20.

5. The pharmaceutical composition of claim 1, wherein the oligonucleotide comprises a 3' inverted thymine base.

6. The pharmaceutical composition of claim 5, wherein the oligonucleotide is selected from the group consisting of SEQ. ID. NO.: 10, SEQ. ID. NO.: 11, SEQ. ID. NO.: 12, SEQ. ID. NO.: 13, SEQ. ID. NO.: 14, SEQ. ID. NO.: 15, SEQ. ID. NO.: 16, SEQ. ID. NO.: 17, SEQ. ID. NO.: 18, SEQ. ID. NO.: 19, and SEQ. ID. NO.: 20.

7. The pharmaceutical composition of claim 1, wherein the oligonucleotide has a chain length of at least about 36 nucleotides.

8. The pharmaceutical composition of claim 1, wherein the oligonucleotide has a chain length of at least about 37 nucleotides.

9. The method of claim 1, wherein the oligonucleotide has a chain length in the range from about 34 nucleotides to about 40 nucleotides.

10. A method of inhibiting replication of a human immunodeficiency virus comprising contacting a cell infected with the human immunodeficiency virus with a oligonucleotide, the oligonucleotide comprising a phosphorothioate inter-sugar linkage, the oligonucleotide having a chain length in the range from about 32 nucleotides to about 50 nucleotides.

11. The method of claim 10, wherein the oligonucleotide has a chain length of at least about 37 nucleotides.

12. The method of claim 10, wherein the oligonucleotide has a chain length in the range from about 34 nucleotides to about 40 nucleotides.

13. The method of claim 10, wherein the human immunodeficiency virus is selected from the group consisting of HIV-1 and HIV-2.

14. The method of claim 10, wherein the oligonucleotide is selected from the group consisting of SEQ. ID. NO.: 5, SEQ. ID. NO.: 6, SEQ. ID. NO.: 7, SEQ. ID. NO.: 8, SEQ. ID. NO.: 9, SEQ. ID. NO.: 10, SEQ. ID. NO.: 11, SEQ. ID. NO.: 12, SEQ. ID. NO.: 13, SEQ. ID. NO.: 14, SEQ. ID. NO.: 15, SEQ. ID. NO.: 16, SEQ. ID. NO.: 17, SEQ. ID. NO.: 18, SEQ. ID. NO.: 19, and SEQ. ID. NO.: 20.

15. The method of claim 10, wherein the oligonucleotide comprises a 3' inverted thymine base.

16. The pharmaceutical composition of claim 15, wherein the oligonucleotide is selected from the group consisting of SEQ. ID. NO.: 10, SEQ. ID. NO.: 11, SEQ. ID. NO.: 12, SEQ. ID. NO.: 13, SEQ. ID. NO.: 14, SEQ. ID. NO.: 15, SEQ. ID. NO.: 16, SEQ. ID. NO.: 17, SEQ. ID. NO.: 18, SEQ. ID. NO.: 19, and SEQ. ID. NO.: 20.

17. The method of claim 10, wherein the oligonucleotide is administered in a final concentration in the range from about 5 nM to about 100 nM.

18. The method claim 10, wherein the oligonucleotide is administered in a final concentration in the range from about 10 nM to about 50 nM.

19. The method of claim 10, wherein the oligonucleotide is administered in a final concentration greater than about 10 nM.

20. The method of claim 10, wherein the oligonucleotide is administered at final concentration greater than about 40 nM.

21. A method of inhibiting syncytia formation caused by a human immunodeficiency virus infection comprising contacting a cell infected with a human immunodeficiency virus with an effective amount of an antiviral composition; the antiviral compositing consisting of an oligonucleotide having at least one phosphorothioate inter-sugar linkage, the oligonucleotide having a chain length in the range from about 32 nucleotides to about 50 nucleotides.

22. The method of claim 21, wherein the oligonucleotide has a chain length of at least about 37 nucleotides.

23. The method of claim 22, wherein the oligonucleotide has a chain length in the range from about 34 nucleotides to 40 nucleotides.

24. The method of claim 21, wherein the human immunodeficiency virus is selected from the group consisting of HIV-1 and HIV-2.

25. The method of claim 21, wherein the oligonucleotide is selected from the group consisting of SEQ. ID. NO.: 5, SEQ. ID. NO.: 6, SEQ. ID. NO.: 7, SEQ. ID. NO.: 8, SEQ. ID. NO.: 9, SEQ. ID. NO.: 10, SEQ. ID. NO.: 11, SEQ. ID. NO.: 12, SEQ. ID. NO.: 13, SEQ. ID. NO.: 14, SEQ. ID. NO.: 15, SEQ. ID. NO.: 16, SEQ. ID. NO.: 17, SEQ. ID. NO.: 18, SEQ. ID. NO.: 19, and SEQ. ID. NO.: 20.

26. The method of claim 21, wherein the oligonucleotide comprises a 3' inverted thymine base.

27. The method of claim 16, wherein the oligonucleotide is selected from the group consisting of SEQ. ID. NO.: 10, SEQ. ID. NO.: 11, SEQ. ID. NO.: 12, SEQ. ID. NO.: 13, SEQ. ID. NO.: 14, SEQ. ID. NO.: 15, SEQ. ID. NO.: 16, SEQ. ID. NO.: 17, SEQ. ID. NO.: 18, SEQ. ID. NO.: 19, and SEQ. ID. NO.: 20.

28. The method of claim 21, wherein the oligonucleotide is administered in a final concentration in the range from about 5 nM to about 100 nM.

29. The method claim 21, wherein the oligonucleotide is administered in a final concentration in the range from about 10 nM to about 50 nM.

30. The method of claim 21, wherein the oligonucleotide is administered in a final concentration greater than about 10 nM.

31. The method of claim 21, wherein the oligonucleotide is administered at final concentration greater than about 40 nM.

32. A method of inhibiting the replication of a human immunodeficiency virus comprising contacting a cell infected with the human immunodeficiency virus with an effective amount of an antiviral composition, the antiviral compositing consisting of an oligonucleotide having at least one phosphorothioate inter-sugar linkage, the oligonucleotide having a chain length in the range from about 32 nucleotides to about 50 nucleotides, and wherein the oligonucleotide is selected from the group consisting of SEQ. ID. NO.: 5, SEQ. ID. NO.: 6, SEQ. ID. NO.: 7, SEQ. ID. NO.: 8, SEQ. ID. NO.: 9, SEQ. ID. NO.: 10, SEQ. ID. NO.: 11, SEQ. ID. NO.: 12, SEQ. ID. NO.: 13, SEQ. ID. NO.: 14, SEQ. ID. NO.: 15, SEQ. ID. NO.: 16, SEQ. ID. NO.: 17, SEQ. ID. NO.: 18, SEQ. ID. NO.: 19, and SEQ. ID. NO.: 20.

33. The method of claim 32, wherein the oligonucleotide has a chain length of at least about 37 nucleotides.

34. The method of claim 32, wherein the human immunodeficiency virus is selected from the group consisting of HIV-1 and HIV-2.

35. The method of claim 32, wherein the oligonucleotide comprises a 3' inverted thymine base.

36. The method of claim 32, wherein the oligonucleotide is administered in a final concentration in the range from about 5 nM to about 100 nM.

37. The method of claim 32, wherein the oligonucleotide is administered in a final concentration in the range from about 10 nM to about 50 nM.

38. The method of claim 32, wherein the oligonucleotide is administered in a final concentration greater than about 10 nM.

39. The method of claim 32, wherein the oligonucleotide is administered at final concentration greater than about 40 nM.

40. A method of inhibiting the replication of a human immunodeficiency virus comprising contacting a cell infected with the human immunodeficiency virus with an effective amount of an antiviral composition, the antiviral compositing consisting of an oligonucleotide having at least one phosphorothioate inter-sugar linkage, the oligonucleotide having a chain length in the range from about 32 nucleotides to about 50 nucleotides, and wherein the oligonucleotide comprises one or more features selected from the group consisting of a 3' hairpin structure a 3' inverted thymine base.

41. The method of claim 40, wherein the oligonucleotide has a sequence selected from the group consisting of SEQ. ID. NO.: 5, SEQ. ID. NO.: 6, SEQ. ID. NO.: 7, SEQ. ID. NO.: 8, SEQ. ID. NO.: 9, SEQ. ID. NO.: 10, SEQ. ID. NO.: 11, SEQ. ID. NO.: 12, SEQ. ID. NO.: 13, SEQ. ID. NO.: 14, SEQ. ID. NO.: 15, SEQ. ID. NO.: 16, SEQ. ID. NO.: 17, SEQ. ID. NO.: 18, SEQ. ID. NO.: 19, and SEQ. ID. NO.: 20.

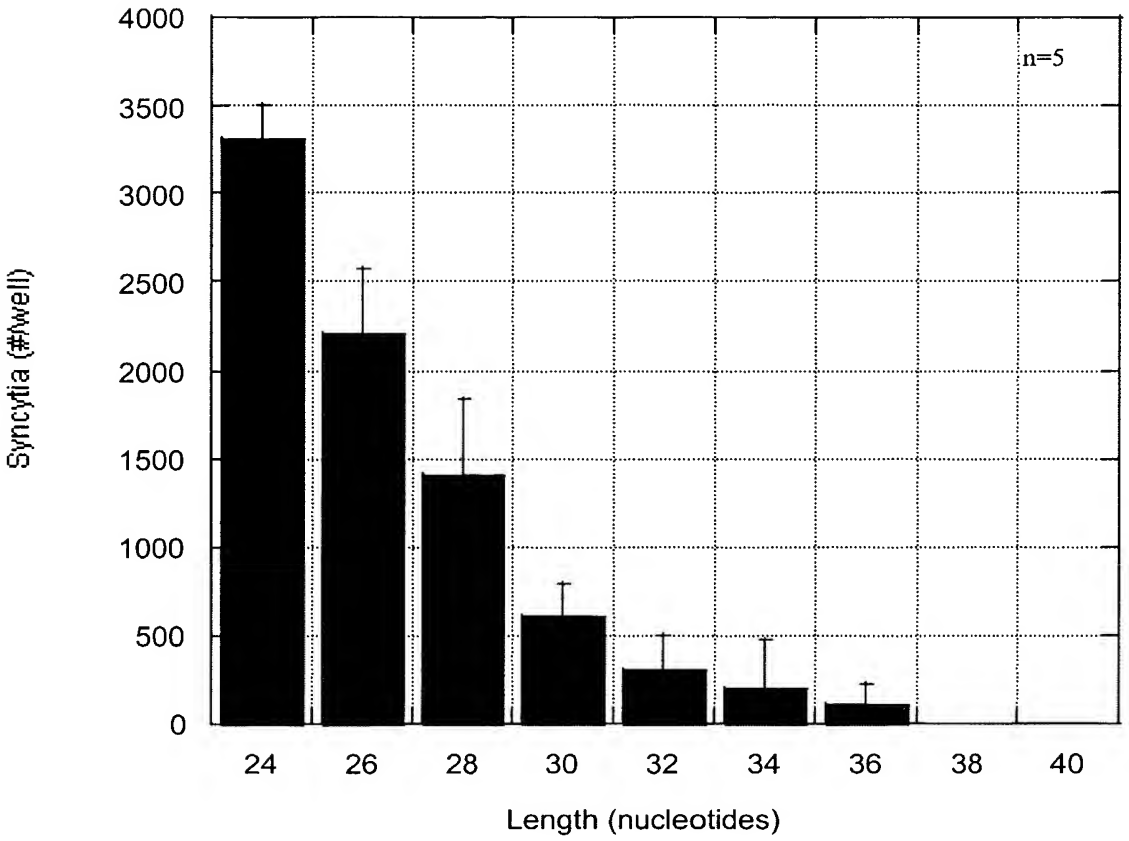
42. The method of claim 40, wherein the oligonucleotide has a chain length of at least about 37 nucleotides.

43. The method of claim 40, wherein the human immunodeficiency virus is selected from the group consisting of HIV-1 and HIV-2.

44. The method of claim 40, wherein the oligonucleotide is administered in a final concentration in the range from about 5 nM to about 100 nM.

45. The method of claim 44, wherein the oligonucleotide is administered in a final concentration in the range from about 10 nM to about 50 nM.

46. The method of claim 44, wherein the oligonucleotide is administered at final concentration greater than about 40 nM.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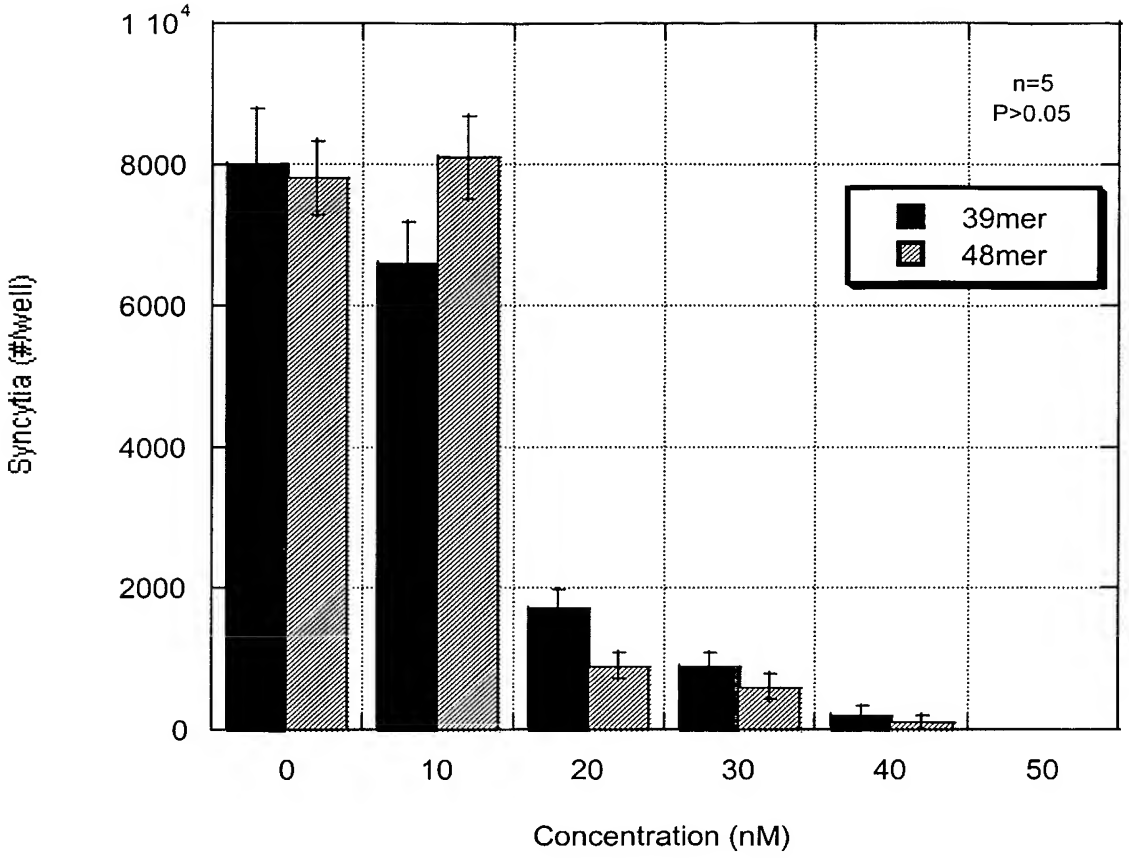
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Figure 2

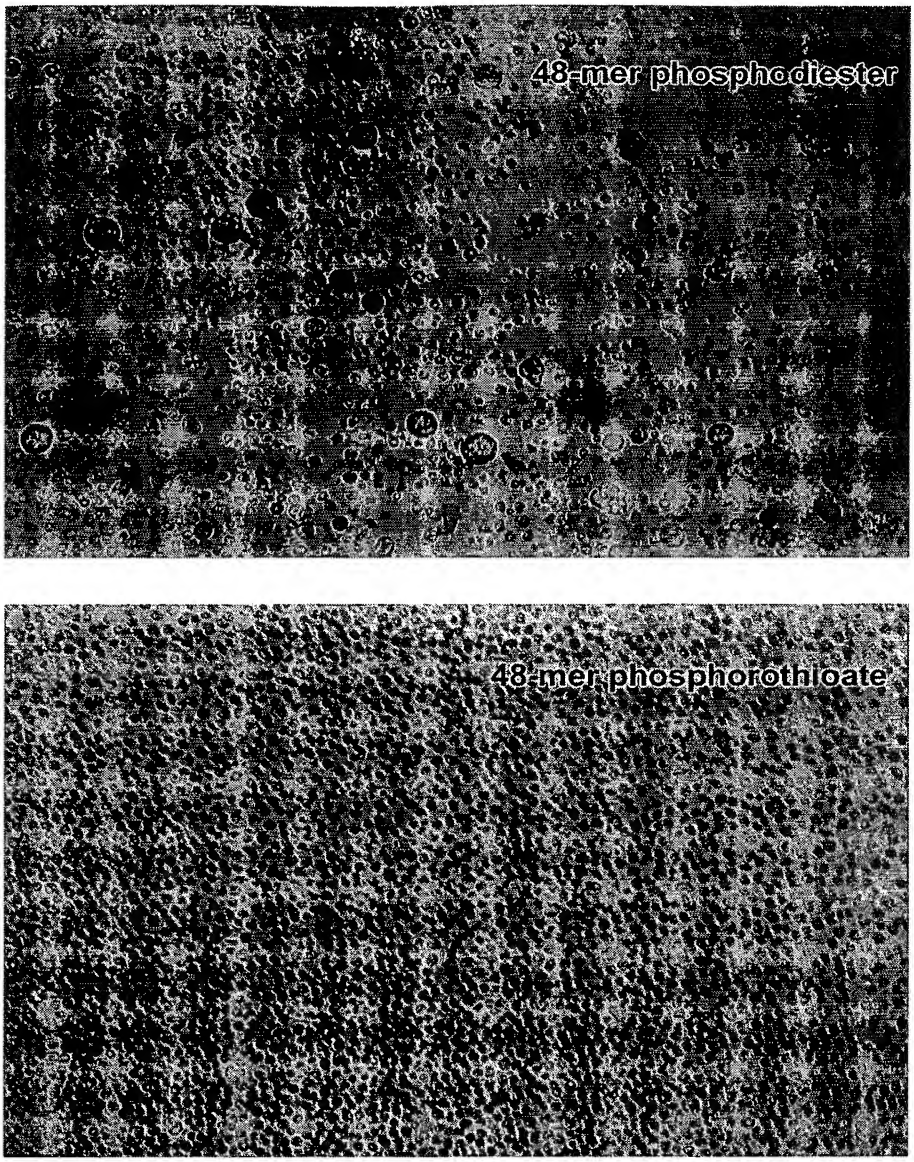

Figure 3

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